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Factors affecting the resistance of house flies to insecticides

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FACTORS AFFECTING THE RESISTANCE OF HOUSE FLIES TO INSECTICIDES

by

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INTRODUCTION

The common house fly, Musca domestica L., may be considered only a nuisance pest to those who do not know its habits. When one learns of its disease-carrying capacity, however, the house fly becomes an enemy to be combatted with all resources. These include the practice of sanitation about the premises to prevent breeding of flies, mechanical exclusion of flies from buildings, and killing as great a proportion of the fly population as possible. The elimination of attractive situations and the use of repellents have also been of value.

Early in this century the only chemicals of value in killing house flies were sodium or potassium arsenite and formaldehyde used as baits, and pyrethrum powder dispersed as a space treatment. Later, pyrethrum extracts were formulated for use as sprays. Efforts were directed toward finding more effective materials to use as sprays. The disadvantages of these early space sprays were that they worked best in closed areas and promised little residual effect.

From 1946 on, DDT and many other residual chemicals came into wide use. These could be applied to leave residual films which would be toxic to flies and many other insects. Also by 1946 much information had been gathered and disseminated about the need for controlling house flies to insure a healthful environment. For this reason there was widespread use of these new residual-type insecticides in fly-control programs. Initial success of the campaigns had the effect of increasing the acceptance and use of this means of control until it became obvious the results were not uniformly satisfactory. When first reports of the failure of DDT to control house flies were made, many reasons were formulated to explain the phenomenon. One of the many explanations was that house flies had developed a resistance toward the action of DDT and therefore were more difficult to kill.

It is known that certain groups of living organisms have built up a tolerance or resistance to environmental factors that have an adverse effect upon the population. In the insects, also, there have been other cases where resistance has developed within a population to the action of an insecticide.

Since house fly control has become very desirable for aesthetic and health reasons, this situation presents a very serious problem. The immediate answer to the problem may lie in finding more potent chemicals and better methods for house fly control. Basically, however, the important consideration is to elucidate why a fly population becomes more resistant to a control chemical. This may allow us to prevent the situation from reoccurring with future practices or at least to be able to predict intelligently what might happen. To do this will require basic research into the bionomics, physiology, and genetics of the house fly.

The purpose of the present investigation is to gain additional information on the hereditary process in the house fly with special reference to the inheritance of resistance to DDT. To do this, laboratory populations have been maintained of a normal strain of *Musca domestica* L. and a strain showing marked resistance to DDT poisoning. Appropriate crosses have been made involving these two strains and the results interpreted in the light of other published findings in this field.

In the course of the investigation, a mutation for white eyes in the house fly was noted and isolated for study. The heredity of this mutation was investigated and is presented as evidence for normal chromosomal inheritance in this species in confirmation of previously published cytological evidence on the chromosomal behavior in *M. domestica*.

REVIEW OF LITERATURE

General

The synthesis of DDT was first reported in the chemical literature by Zeidler (1874) who was studying combinations of chloral with bromo- and chloro-benzenes. In brief, Zeidler's DDT synthesis consisted of reacting chlorobenzene and chloral in the presence of concentrated sulfuric acid. The crude crystals obtained in this reaction were separated, washed with water, and recrystallized from ethyl alcohol. The product obtained consisted of quite pure 2,2 bis-(p-chlorophenyl)-1,1,1-trichloroethane (m. p. 105° C.). The pure p,p'-isomer has a melting point of 108.5°-109° C.

No further use was found for this chemical until its insecticidal properties were discovered in the autumn of 1939 by Dr. Paul Müller and co-workers working in the Basle, Switzerland laboratories of J. R. Geigy Company. The story of this discovery and the following insecticidal development of DDT has been recounted by several authors, including West and Campbell (1952). During the early development of DDT, Switzerland was surrounded by countries at war, therefore the product was not introduced to the United Kingdom and the United States until late in 1942. By this time much was already known concerning the compound. As the latter countries were also at war at this time, the development and use of the material proceeded under high priority and some secrecy. First full-scale use of DDT outside Switzerland was in December, 1943 at Naples, Italy to combat an epidemic of louse-borne typhus fever. Meantime research had been going on in the United States as reported by Annand *et al.* (1944). Some of these experimental trials were on a limited field basis.

Resistance to insecticides has been the subject of several reviews. Quayle (1943) has reviewed the subject to the advent of DDT. Recent reviews include those by Monro (1949), Babers (1949) and Babers and Pratt (1951, 1952). In more restricted reviews, Hess (1952) covered resistance in insects of medical importance while Decker and Bruce concentrated on house fly resistance. Chadwick (1952) covered mainly physiological studies of DDT resistance in insects. The points of view of many of the leaders in this field were brought together in the report of the Conference on insect resistance and insect physiology (National Research Council, 1952).

Research on the problem of house fly resistance to DDT has taken three main directions. These are toward new methods of control, the physiological basis of resistance, and the genetical basis of resistance. This review will specifically deal with those articles reporting on the genetical basis of resistance and the necessary cytological background.

Cytological

Stevens (1908) correctly determined the diploid chromosome number in *Musca domestica* L. to be $2n = 12$. This included five pairs of autosomes and an unequal pair of heterochromosomes which were the sex chromosomes. There was an equal pair of large chromosomes in the female and an unequal pair (large and small) in the male. This observation was later confirmed by Metz (1916).

Of special interest to this problem is the type of division which takes place in each chromosome pair during meiosis. White (1950) arranged the Diptera into phylogenetic groups based on cytological evidence. In this grouping the "higher" Diptera, which included all families of the Brachycera, had lost the phenomenon of chiasma formation in the male but all chromosomes had pairing segments, including X and Y. Obviously every species had not been examined to verify this grouping but all cytological evidence cited supported it. Since *Drosophila* and *Musca* both are included in the Brachycera in White's classification, it may be expected that, as in *Drosophila*, chiasma formation and crossing over occur in the house fly female but not in the male.

The most recent work on the chromosome behavior in *Musca domestica* was by Perje (1948a, 1948b). The five pairs of autosomes were found to be metacentric and each had two long chromosomal arms. The chromosome pairs varied enough in size and shape so that they could be easily identified. The X and Y chromosomes were described as being heterochromatic to a greater extent than in *Drosophila melanogaster* Meigen. Perje assumed that as heterochromatic parts in the chromosomes usually lacked genes, it was likely that there would be only a few or perhaps no sex-linked genes in *Musca domestica*. As yet no sex-linked genes have been reported, but the number of genes reported affecting *M. domestica* is very small and none has been assigned to a chromosome.

Genetical

Little work had been done on the genetics of *M. domestica* prior to the discovery of DDT resistant house flies. In one case, however, Barber

(1948) isolated two morphologically different lines and perpetuated them in pure cultures. These were designated as a lethal line because a small percentage of the pupae hatched and a flat line indicating flattened pupae. The larvae in the lethal line failed to retract the mouth parts before pupating. Although crosses were made with other lines and the progeny observed, no estimate of the genes involved could be made.

Genetical changes in DDT resistant house flies have been studied along with physiological aspects of the problem. D'Alessandro _et al._ (1949) used knockdown time as a scale for resistance. In one cross of a resistant female and a susceptible male the F_1 generation varied widely as did the F_3 generation. They concluded that the phenomenon of DDT resistance was caused by an extremely varied hereditary character.

Bruce (1950) developed and determined the resistance of house fly strains by topically applying acetone solutions of DDT to the thorax of females. Mass crosses between resistant and non-resistant strains did not eliminate resistance but merely diluted it. The level of DDT resistance was somewhat intermediate between the resistance of the two parents. Reciprocal crosses produced similar results indicating that both the male and female flies carry the resistance characters. It was further stated by Bruce and Decker (1951) that there was little reason to believe that DDT-resistant strains of different origins were genetically identical. Still later, Bruce (1954) speculated that the most important genetical factor in DDT resistance was dominant but that other factors of lesser importance were also involved.

The first report of simple Mendelian inheritance in connection with

DDT resistance in house flies was by Harrison (1951, 1953). This work was carried on at the London (England) School of Hygiene and Tropical Medicine with two strains of resistant flies from Italy and Sardinia. Individual crosses were made by separating the pupae into individual vials and pairing the adults after emergence from the pupae. The flies were scored by counting the time for knockdown during continuous exposure to a DDT-treated surface. In reciprocal crosses between the Italian strain and a susceptible strain, the F_1 generation was similar in knockdown time to the susceptible strain. In the F_2 generation, resistance to knockdown segregated out in a 1 to 3 ratio. Thus it was assumed that a single gene controlled knockdown with the gene for susceptibility being dominant. This gene was not present in the Sardinian strain.

However, when DDT in mineral oil was applied topically to the thorax of individual flies and the resultant mortality recorded, both the Italian and Sardinian strains were equally resistant to DDT. In preliminary experiments individual crosses between the Italian and a susceptible strain produced a homogeneous F_1 generation with resistance being intermediate between that of the parental strains. The F_2 generation was considerably more heterogeneous. These results were interpreted as indicating a multiple factor inheritance with no evidence of cytoplasmic inheritance or sex linkage since reciprocal crosses gave similar results. It should be emphasized that in this case inheritance of resistance to knockdown was entirely different from that of resistance to mortality.

Similar results were reported for resistance to knockdown by Keiding (1951). Using a resistant strain from Denmark and a technique similar to that used by Harrison, resistance was found to be inherited by a single gene with susceptibility prevailing but not fully dominant over resistance.

In Italy, La Face (1952a, 1952b) was unable to separate any single factor responsible for resistance by crossing a normal with a DDT- and chlordane-resistant strain of house flies. Using a single pair each generation, a line was propagated for 15 generations. Several pairs were started from each generation to test the progeny. They concluded that resistance was controlled by many factors in a complex system.

Maelzer and Kirk (1953) in Australia have made a very careful study of the genetical factors responsible for resistance in the Multi-X strain received from Illinois and previously reported by Bruce and Decker (1951). These authors used as a criterion of effect both knockdown and mortality following topical applications of acetone solutions of technical DDT. Knockdown alone was used following constant exposure to a film of DDT in a 1000 ml. beaker. After making crosses and obtaining eggs the resistance of each parent was determined by checking the time for knockdown following a dose of 8 micrograms of DDT per fly. By this method it was possible to divide the resistant parents of each sex into two classes, i.e., those of moderate resistance and those of strong resistance. With appropriate crosses between susceptible flies and these two types of resistant flies, they concluded that the intermediate resistant flies possessed many genic factors for resistance which they could not separate. The strongly resistant flies, however, had a single dominant gene involved which also had the effect of seriously reducing fertility. The authors postulate that this reduced fertility would reduce the frequency of this particular gene in a population in the absence of strong selection for DDT resistance.

In one of the most thorough investigations into inheritance of DDT resistance in the house fly yet reported, Norton (1953) examined several resistant strains. Adult flies were exposed to vaporized DDT within an exposure chamber. Dosage was determined by time of treatment. Strains of resistant flies from Illinois, California, New Jersey, New Hampshire, and a susceptible laboratory strain were used. The percentage mortality at a constant predetermined dosage was used to evaluate the susceptibility of each strain and the progeny of crosses. No difference in tolerance to DDT could be detected between progeny of paired crosses and mass crosses.

In mass crosses between a resistant strain and a susceptible strain the F_1 generation was about midway between the level of resistance shown by the parental strains. This level was maintained without appreciable change through ten ensuing generations. Crosses between resistant strains indicated a rather uniform pattern of tolerance transmission. Each of the four resistant strains exhibited a different resistance level. When resistant strains were crossed, the degree of resistance of the F_1 generation fell between the parents, which again was maintained throughout ten generations.

When the progeny of a cross were backcrossed successively to the more resistant parent the offspring became progressively more resistant. When backcrossed to the susceptible parent the level of resistance was gradually lowered but did not completely disappear during ten generations of crossing.

When the Illinois and California strains were mated with the laboratory strain, the level of resistance in reciprocal crosses was not identical. In all these crosses, the progeny were more like the female parent. On the other hand, reciprocal crosses were alike between either the New Jersey or New Hampshire strain and the laboratory strain. The differences noted in reciprocal crosses were not definite enough to indicate either sex linkage or cytoplasmic inheritance.

In somewhat similar tests Pimentel et al. (1954) also used reciprocal crosses. The level of resistance was determined by exposing the flies in DDT-treated test cages continuously for 12 hours. At the end of this period, all flies knocked down were counted as dead. The non-resistant strain was the standard NAIDM (now CSMA) stock for 1949. The resistant strain was collected in the field from a population known to be resistant to DDT. This resistant field strain was subjected to severe selection for 25 generations by allowing the flies to emerge into cages treated with heavy doses of DDT. Finally, during the last three generations, only those flies that pupated during the last three days of the larval period were selected for exposure to the DDT. Earlier Pimentel et al. (1951) had reported that late pupating larvae produced more resistant flies than the earlier ones in the same culture. This mass selection failed to produce flies homogeneous for resistance since resistance decreased when 20 additional generations were reared without selection.

Reciprocal mass crosses were made between the selected DDT-resistant strain and the non-resistant strain. The progeny were somewhere between the two parental strains. Through the fifth generation there were no individuals either as resistant or non-resistant as the parents. The progeny of these two crosses also lacked homogeneity for resistance since resistance was lost after 20 generations of no exposure to DDT.

The resistance of the progeny was higher and the larval period was longer when the female flies were from the resistant strain as compared to the reciprocal cross. The influence of the female on the resistance of the progeny was not sex linked since the daughters were affected in the same manner as the sons.

In attempting to explain the differences resulting from reciprocal crosses as also reported by Norton (1953) and Pimentel (1954), an entirely different means of inheritance of resistance to DDT was postulated by Johnston et al. (1954) who crossed two resistant strains with a susceptible strain. After establishing that single pair crosses produced similar results to mass crosses, all crosses were made en masse. DDT was applied in acetone solution to the thorax.

In the F_1 generation of reciprocal crosses between a resistant strain and a susceptible strain no evidence for sex linkage was observed. There was no significant difference in resistance of flies produced by the two types of matings. However, from studies of the resistance in F_2 generations and backcrosses it was deduced that the factor responsible for resistance to DDT in both resistant strains was carried in the cytoplasm. It was stated that this cytoplasmic factor was particulate in character and was controlled by one or more nuclear genes since there was a one generation lag in loss of resistance. Further, the actual presence of DDT was necessary for the active reproduction of these particles. Also a few of these particles could be transmitted in a small amount of cytoplasm with the sperm. Finally the length of larval life directly influenced the amount of resistance developing by allowing the cytoplasmic particles more time to develop. This was true whether the larvae were inherently late pupating or were retarded by low temperature during development. The authors emphasized that either different strains of resistant flies behaved differently in mode of inheritance or that different environmental conditions drastically influenced the results.

MATERIALS AND CULTURE METHODS

Chemical

The DDT used in this investigation was pure 2,2-bis-(p-chlorophenyl) 1,1,1-trichloroethane. The pure p,p' isomer of DDT was obtained from technical DDT of unknown origin by crystallizing twice from 95 per cent ethyl alcohol as outlined by Fleck et al. (1945). The crude technical material was dissolved in boiling 95 per cent ethyl alcohol, filtered, the filtrate cooled to 30° C., filtered again, and the crystals thus obtained were dried. The melting point of the purified compound was 108-109° C. The pure compound melts at 108.5-109° C.

Biological

The house fly, *Musca domestica* L., was used altogether in this investigation. Two strains, varying in their ability to survive after treatment with DDT, were maintained as separate cultures. The susceptible strain (S) was cultured from pupae from the 1952 NAIDM (now CSMA) strain maintained by the Rohm and Haas Company, Philadelphia, Pennsylvania. A resistant strain (R) was started with eggs collected from female flies captured in 1952 at the Clemson College dairy barn, Clemson, South Carolina. These barns had been treated with DDT and other residual-type fly control materials since 1946.

A culture of white-eyed flies was started by collecting eggs from a single gravid white-eyed female discovered in the DDT resistant colony. The adults reared from these eggs were all wild type in appearance. This group was allowed to mate at random. The following generation contained both male and female flies with white eyes. Males and females with white eyes were isolated in a separate cage soon after hatching. Each succeeding generation contained all white-eyed flies. The line was continued for nearly two years, 24 generations, without contamination from outside flies although maintained in the same room with the wild-type strains.

All fly cultures were reared at 27° C. in a room provided with artificial light only. The adults were maintained in screened wire cages with wooden ends and bottoms. A cloth sleeve in one end provided access to the interior of the cage. The adults were fed commercial sugar (sucrose), fat free milk, and water. The larvae were reared in CSMA medium moistened with a suspension of live yeast cells and malt extract. Complete directions and sources of material are given in the Soap and Sanitary Chemicals Blue Book (Peet-Grady Method, 1954). Eggs from mass cultures were collected by introducing a dish of the larval food into the cage and leaving it until eggs were deposited in it. A random sample of these eggs was used to start new cultures.

In order to make crosses between strains it was necessary to establish a method to obtain virgin individuals for mating. One way to insure virgin flies was to place individual pupae in separate vials for hatching, then introduce the flies by sex into a rearing cage as desired. This system proved cumbersome if extensive tests were planned. An alternative method was tried which followed the commonly used system in mating

Drosophila. The adults were allowed to emerge from the pupae into a small cage with an entrance sleeve where they could be captured individually in a vial. By moving the unhatched pupae to a fresh cage, adults could be obtained one or two hours later.

It was necessary to determine the age at which adults would be sexually mature enough to mate. By observation it was determined that mating began about 24 hours after introduction of hatching pupae into a cage. Thus it was felt that a maximum aging period of 12 hours might be safely used. In preliminary tests the sexes were transferred as soon after hatching as possible. With the discovery of the white-eyed flies, however, it was possible to detect non-virgin matings with wild-type, especially, if females with white eyes were used. Since no non-virgin matings were detected in any of these crosses it was felt that the original observations were correct. It should be emphasized that environmental conditions might vary the time required for newly hatched adults to become sexually mature enough to mate.

Single pair crosses were carried out by capturing virgin females in a small glass vial and confining each in a cloth-covered one-quart mason glass jar with the proper male. Food was supplied by adding separately a small lump of sugar and milk in a small dish fashioned from aluminum foil. Eggs were collected from these pairs by introducing into the jar a 25 X 95 mm glass shell vial containing the larval medium. When by inspection it was determined that an egg cluster had been deposited, the medium was transferred to a wide-mouth, one-pint glass jar one half full of the larval medium. A female house fly deposited about 100 eggs per cluster.

A slight modification was made in the usual larval food since with small numbers of larvae the mass tended to remain compact so that the larvae collected in one spot allowing the rest of the material to ferment and mold. By adding a small amount of additional inert material such as oat hulls or chaff with the necessary additional water, a medium was produced which was porous enough to allow the larvae to migrate throughout. Using this method, pupae were produced comparable to those from the larger cultures. Progeny from these single pair matings were allowed to emerge into small screen cages (8 X 8 X 12 inches).

It has previously been recorded (Tate, 1948) that ordinary precautions were not enough to prevent contamination of a strain of blow flies when reared in open screen cages. Knipe and Fringe (1953) also reported that female house flies would drop eggs through a screen onto suitable larval food and could extend the ovipositor at least 12 mm to reach such food through a screen. Therefore, all escaped flies in the rearing room were poisoned with a bait containing two per cent formaldehyde in milk diluted one half with water.

EXPERIMENTAL PROCEDURE

The strain of white-eyed flies was mated using single pairs in the quart jars and also with ten of each sex in larger screen cages. The purpose of these matings was threefold:

(1) to determine the genetical basis of the white-eyed flies,

(2) to check with an easily identified mutant whether or not there were any unusual features in inheritance in house flies,

(3) to determine if any genetical contamination occurred unnoticed.

Reciprocal crosses were made and the F_1 generation was carried through the F_2 generation. In these crosses and all other crosses the F_2 generation of progeny was obtained by mating individuals that had identical parents. Individuals secured from mass crosses were never used for future matings. The F_1 generation flies from each reciprocal cross were also crossed back to both male and female white-eyed flies. The phenotype of every individual produced over the entire range of hatching was recorded.

For testing purposes, 3 to 5 day old flies were anesthetized with CO_2 (Williams, 1946), then separated by sex into lots of five and placed in petri dishes. The flies were then allowed to recover and were held for testing. The DDT was applied to individual flies by topical application of an acetone solution to the dorsal surface of the thorax. To do this, the flies were again anesthetized with CO_2, then picked up individually by the wing with a pair of tweezers with blunt ends. The fly was held to the tip of a special micro-pipette and 0.002 ml. (2 microliters) of acetone was transferred to the fly by contacting the drop. The fly was returned to the dish until the entire lot was treated, then food was added and the dish stored at 27° C. After 24 hours, mortality among the flies was recorded. Those flies which could not stand or walk were counted as dead. This criterion was adopted because of the difficulty in judging when all movement had ceased and because it was determined that flies not able to walk after 24 hours at this temperature died anyway.

The micro-pipette was constructed from a 0.1 ml. serological pipette graduated in 0.001 ml. The portion above the graduations was bent into a U-shape and attached to a small reservoir of mercury, the height of which could be adjusted with a screw-type plunger. The plunger could be rotated with the one hand to change the height of the mercury column and thus raise or lower the acetone column in the lower part of the pipette. The mercury was never allowed to enter the portion of the tube calibrated for measuring the volume of DDT-acetone solution. The main advantage of this pipette over the 0.25 ml. tuberculin syringe with attached steel needle usually used is that the all-glass pipette can be easily decontaminated by rinsing with acetone several times and the change from one concentration to another easily made.

The dosage of actual DDT per fly was adjusted by varying the concentration of DDT in the acetone solution. The final dilution was expressed in micrograms of DDT per 2 microliters of solution which was equivalent to micrograms per fly since this volume administered always remained the same. The same dosage of DDT was applied to both males and females but in later comparisons the two sexes were always considered separately. It was determined that neither the CO_2 nor the amount of acetone used caused any mortality among the flies.

In working with single pair crosses of house flies one is limited in the number of progeny available for testing. Usually about 50 healthy flies were available as the result of each egg cluster deposited. For this reason it was impossible to characterize completely the resistance of each group of progeny in terms of actual mortality produced by several different dosages in replicated tests. Instead, the mortality to groups of five flies of each sex was determined for not over four different dosages.

Reciprocal crosses were made between the susceptible and the resistant strains of house flies using single pairs. An F_2 generation was reared with some of the resulting flies. Other F_1 flies were backcrossed to the susceptible strain. The response to DDT was determined for each group of adults, however, untreated flies were always used as parents for new matings.

RESULTS

Analysis of the Factor for White Eyes

The method of origin suggested that white eyes might be controlled by a single recessive gene which was not sex linked. A series of planned crosses were then made to test this hypothesis. The factor for the white-eyed condition shall here be designated as (w) while the wild type allele shall be designated as (+). First, a white-eyed female was mated with a wild-type male. The F_1 progeny of this cross were all wild type. The reciprocal cross produced the same results. Next an F_2 generation was reared from each cross. In addition, F_1 males and F_1 females from each original cross were backcrossed to white-eyed females and white-eyed males, respectively. A summary of the results of these crosses is shown in Table 1. The results of each individual cross are given in Table 6 and Table 7 in the Appendix.

According to theory a single completely recessive gene should not show in the phenotype of the F_1 generation but should reappear in the F_2 phenotype in a 1 to 3 ratio. When the heterozygote is backcrossed to the homozygous recessive the homozygous phenotype should reappear in a 1 to 1 ratio. The evidence presented here supported this expectation in both cases. An adjusted value of chi-square was calculated in each case as a measure of the deviation of the expected ratio from the postulated ratio. In five of the six cases tested the variation was not

Table 1. Segregation of white eye (w) following crosses with the wild type (+) in *M. domestica*.

Mating	Generation[a]	Observed number w	Observed number +	Expected number[b] w	Expected number[b] +	X^2 value[c]
+ ♂ X w ♀	F_2	368	1198	391.5	1174.5	1.80
w ♂ X + ♀	F_2	366	1210	394	1182	2.56
(w ♂ X + ♀) ♂ X w ♀	BC_1	379	452	415.5	415.5	6.24
w ♂ X (w ♀ X + ♀) ♀	BC_1	316	288	302	302	1.21
(+ ♂ X w ♀) ♂ X w ♀	BC_1	113	99	106	106	0.80
w ♂ X (+ ♂ X w ♀) ♀	BC_1	250	208	229	229	3.77

[a]The F_1 generation contained only wild type individuals.

[b]Based on an expected ratio of 1:3 in the F_2 and 1:1 in the back-cross to the recessive parent.

[c]Adjusted chi-square calculated using the formula $X^2 = (|X - m_1| - 0.5)^2/m_1 + (|X - m_2| - 0.5)^2/m_2$ as given in Snedecor (1946).

great enough to indicate other than sampling variation from the genetic ratio. In one case $\left[(w ♂ X + ♀) ♂ X w ♀ \right]$ the chi-square value of 6.24 indicated only about one chance in a hundred that the sample was drawn from a population in which the true ratio was 1 to 1. The bulk of the evidence, however, supported the original hypothesis stated above.

The Method of Inheritance of DDT-Resistance

When level of resistance in the two parental strains was determined, there was no overlap of the two populations as may be seen from the results given in Table 2. In the susceptible strain mortality increased with an increase in dosage. On the other hand, mortality in the resistant strain reached a maximum and additional increases in dosage failed to produce any further increase in mortality. This was especially true in the female. In addition it may be stated that the original strain was cultured in the laboratory for nearly a year, 12 generations, with no noticeable loss of resistance.

A total of 11 crosses was made involving a resistant male and a susceptible female. The fractions killed when the progeny were treated with various doses of DDT are shown in Table 3. In this table and in Table 4 the mating number is simply a number assigned for identification. In this case resistance was introduced into the cross through the male. The level of DDT resistance was fairly high in both males and females of the F_1 generation. Thus the resistance was transmitted through the male to both sexes of progeny. A linkage with sex would be indicated by susceptible F_1 males regardless of the resistance in the F_1 females since

Table 2. Mortality of two strains of house flies when DDT was applied topically in acetone solution.

Dosage Micrograms per fly	Male Dead	Male Total	Male Percent	Female Dead	Female Total	Female Percent
		Clemson (R) strain				
160	4	5	80	1	5	20
80	5	5	100	1	5	20
40	4	5	80	3	5	60
20	21	43	49	7	23	30
10	4	35	11	1	16	6
5	1	5	20	0	4	0
2.5	0	5	0	0	5	0
1.25	0	12	0	0	6	0
Check	0	16	0	0	8	0
		NAIDM (S) strain				
0.60				10	10	100
0.30				19	20	95
0.20	6	6	100	22	22	100
0.17	6	6	100	13	16	81
0.14	6	6	100	16	31	52
0.12	6	6	100	3	14	21
0.10	5	6	83	10	28	36
0.08				0	10	0
0.04				0	10	0
Check	0	6	0	0	33	0

Table 3. Mortality of progeny from (resistant male X susceptible female) when treated with DDT.

Mating number	Generation	Micrograms per fly	Fraction killed	
			Male	Female
2	F_1	200	5/5	5/5
		20	5/5	3/6
		2	...	0/5
		0.2	3/5	...
4	F_1	200	...	2/5
		20	3/5	0/5
		2	0/5	0/5
		0.2	0/5	...
6	F_1	200	4/5	5/5
		20	5/5	1/6
		2	0/6	...
		0.2	...	...
8	F_1	200	5/5	4/5
		20	5/5	3/7
		2	2/5	...
		0.2	...	...
10	F_1	200	3/5	3/5
		20	3/5	2/5
		2	1/5	0/5
		0.2	...	...
	F_2	200	3/5	4/5
		20	2/5	1/5
		2	2/5	0/5
		0.2	...	...
12	F_1	200	5/5	1/5
		20	1/5	0/5
		2	0/5	0/5
		0.2	...	...
	F_2	200	3/5	4/5
		20	2/5	1/5
		2	2/5	0/5
		0.2	...	...

Table 3. (Continued)

Mating number	Generation	Micrograms per fly	Fraction killed	
			Male	Female
14	F_1	200	4/5	0/5
		20	0/5	0/4
		2	0/5	0/5
		0.2	...	...
16	F_1	200	5/5	5/5
		20	5/5	5/5
		2	1/5	2/5
		0.2	...	...
	F_2	200	5/5	1/3
		20	5/5	1/5
		2	0/5	2/5
		0.2	...	...
18	F_1	200	...	...
		20	5/5	4/5
		2	2/5	0/5
		0.2	0/5	0/5
20	F_1	200	...	No
		20	5/5	female
		2	1/5	progeny
		0.2	0/5	
22	F_1	200	4/5	No
		20	1/5	female
		2	0/5	progeny
		0.2	...	

Table 4. Mortality of progeny from (susceptible male X resistant female) when treated with DDT.

Mating number	Generation	Micrograms per fly	Fraction killed Male	Fraction killed Female
1	F_1	200	...	2/5
		20	2/4	0/5
		2	0/5	0/5
		0.2	0/5	...
3	F_1	200	...	4/4
		20	5/5	5/5
		2	4/5	4/5
		0.2	3/5	...
5	F_1	200	...	2/5
		20	2/5	0/5
		2	2/5	0/5
		0.2	0/5	...
7	F_1	200	...	4/5
		20	1/4	0/4
		2	0/5	0/4
		0.2	0/5	...
9	F_1	200	4/5	1/5
		20	2/5	0/4
		2	0/4	0/5
		0.2	...	...
11	F_1	200	1/4	1/5
		20	2/5	2/5
		2	...	...
		0.2	...	...
13	F_1	200	5/5	4/5
		20	3/5	0/5
		2	0/4	0/4
		0.2	...	...
	F_2	200	5/5	5/5
		20	4/5	3/5
		2	3/5	2/5
		0.2	...	...

Table 4. (Continued)

Mating number	Generation	Micrograms per fly	Fraction killed	
			Male	Female
17	F_1	200	5/5	5/5
		20	4/5	3/5
		2	4/5	0/5
		0.2	...	...
	F_2	200	5/5	4/5
		20	3/4	3/5
		2	3/5	1/5
		0.2	...	...
19	F_1	200	5/5	5/5
		20	5/5	4/5
		2	0/5	2/5
		0.2	...	...
	F_2	200	3/5	5/5
		20	3/4	4/5
		2	3/5	1/5
		0.2	...	...
21	F_1	200	5/5	2/5
		20	0/5	0/5
		2	0/5	0/5
		0.2	...	...
23	F_1	200	...	...
		20	5/5	5/5
		2	2/5	1/5
		0.2	0/4	0/5
23	BC_1 F_1 ♂ X S ♀	20	...	4/5
		2	...	2/3
		0.5	7/10	4/10
		0.1	2/5	0/5
	BC_2 S ♂ X BC_1 ♀	20	5/5	5/5
		2	5/5	5/5
		0.5	5/5	5/5
		0.1	5/5	1/5
	BC_1 F_1 ♂ X S ♀	20	0/5	3/5
		2	3/5	1/4
		0.5	3/5	3/5
		0.1	0/5	0/5

Table 4. (Continued)

Mating number	Generation	Micrograms per fly	Fraction killed	
			Male	Female
23	BC_2	20	5/5	2/5
	S ♂ X BC_1 ♀	2	5/5	0/5
		0.5	3/5	1/5
		0.1	1/5	2/5
	BC_2	20	...	...
	S ♂ X BC_1 ♀	2	4/5	4/5
		0.5	3/5	2/5
		0.1	2/5	3/5
	BC_1	20	5/5	no
	F_1 ♂ X S ♀	2	3/5	female
		0.5	0/5	progeny
		0.1	2/5	
	BC_1	20	4/5	4/5
	F_1 ♂ X S ♀	2	4/5	3/5
		0.5	4/5	1/5
		0.1	3/5	0/5

XY males receive their X chromosome from the mother. Such was not the case. In two F_1 cultures (20 and 22) no females appeared in the adults so females could not be tested. Three F_2 generation cultures were reared for testing also. The level of resistance in these was maintained about equal to that in the F_1 generation (Table 2).

When the reciprocal of the original cross was made, the results were very similar. Here resistance was contributed by the female in the cross susceptible male to resistant female. The results of 11 such crosses are shown in Table 4. In addition three of the F_1 cultures were continued to the F_2 generation for testing. This time resistance was transmitted to both sexes of progeny through the female and this resistance was maintained in the F_2 generation as before. When the F_1 generation of this cross was backcrossed to a susceptible female, however, the resistance level dropped considerably. These progeny were crossed to a susceptible male to produce a still lower resistance level, in fact, approaching that of the original susceptible strain. One of the backcross cultures from cross number 23 failed to produce females as had been noted before in the F_1 generation of number 20 and number 21.

The average level of resistance in each group of the F_1 progeny was calculated by summing the fractions killed for all tests using 20 micrograms per fly. This dosage was chosen because it produced an intermediate mortality. These totals are compared in Table 5 to see if there is any significant difference in the resistance level of the progeny from the two original crosses.

Table 5. Comparison of mortality in the F_1 progeny from reciprocal crosses using 20 micrograms of DDT per fly.

Sex	Cross R ♂ X S ♀ Dead	Total	Percent	Cross S ♂ X R ♀ Dead	Total	Percent	χ^2 values
Male	37	55	67.3	31	53	58.5	0.58[a]
Female	20	48	41.7	19	53	35.8	0.16[b]

[a]Degrees of freedom = 1 P = 0.45

[b]Degrees of freedom = 1 P = 0.69

The mortality of both males and females was higher in the F_1 progeny from resistant male X susceptible female as compared to the cross which involved a resistant female. A resistant female tended to transmit slightly more resistance to her progeny than a resistant male. The level of resistance was not statistically significant. There was no reason either to believe that any biological significance should be placed on this slight difference.

DISCUSSION

The results of the experiments with the white-eye mutation indicate nothing unusual in chromosomal inheritance in the house fly. The cytological evidence previously published by Stevens (1908), Metz (1916), and Perje (1948a, 1948b) on the behavior of the chromosomes during meiosis also point to the same conclusion. This is the first time, so far as the writer knows, that a definite morphological change in Musca domestica has been found to be associated with a single Mendelian factor. It would be of great advantage to the workers using house flies in resistance studies if more mutant genes could be isolated and maintained for use.

The house fly has been considered to be fairly free of observable gene mutations. This may not be the case as two abnormalities were observed during this investigation in addition to the main aberrant strain possessing resistance to the action of DDT. The factor for white eye was isolated and studied but the other case of cultures containing only males remained unsolved. A culture was considered to be all-male if no females were produced over the entire range of hatching. The males were fertile but when crossed with various females the situation did not reoccur. The case was not followed further. In all, five all-male cultures showed up in 37 matings involving resistant and susceptible flies. A similar occurrence in Drosophila melanogaster was reported by Gowen (1947). In that case a female produced only male progeny if she received from her father a third chromosome gene at locus 31. Thus when this gene was heterozygously present in a female all of the XX eggs died but XY eggs were unaffected whether or not they carried the gene. It is not known that the case in the house fly was determined by a similar gene but it is indicated that the phenomenon may be gene controlled.

The white-eye mutation proved useful in verifying the validity of the techniques used for isolating females and the subsequent rearing of isolated families. It would be very difficult to prove contamination in crosses that involved only resistance and susceptibility to DDT. On the other hand non-virgin, white-eyed females when mated to wild-type males could easily be detected by the phenotype of the progeny. By using the technique of separating the sexes soon after hatching, the extra work involved in isolating individual pupae can be avoided provided strict care is exercised.

In the study of the behavior of resistance in inheritance it is not easy to determine the approximate resistance level of any individual fly. Some workers have approached the situation by measuring the time for knockdown following exposure to the poison. This is a convenient way to evaluate each individual but as pointed out by Harrison (1953) resistance to knockdown is not necessarily correlated with resistance to final mortality. Maelzer and Kirk (1953) used a combination of knockdown and mortality following topical applications of DDT in acetone. In their resistant strain knocked down flies did not recover so the observations were valid. The technique would not work if many knocked down flies eventually recovered. Also the rate of knockdown does not necessarily indicate the theoretical amount of insecticide needed to kill each fly.

The method has practical application, though, since rate of inactivation is very important in determining control when the replacement rate is high enough to be important.

Another factor to be considered is the method of application of the toxicant. Allowing the fly to remain on a treated surface is most like the situation encountered in control work. Still there is no knowledge of the amount of material picked up by each individual or the amount of variation between individuals. The same situation applies to the method used by Norton (1953) of vaporizing the DDT. When DDT is applied directly to the fly by topical application the amount added to each fly is known. It has been pointed out by Busvine (1951a, 1951b), however, that at high dosage in acetone, DDT does not penetrate well. In fact, as the concentration of DDT in acetone is increased the percent absorbed decreases. Thus the apparent resistance level is greatly magnified as resistance approaches high levels. On the other hand when the DDT is dissolved in mineral oil the ratio (resistant to normal) is much lower. The reason pointed out is that acetone evaporates leaving crystals of DDT which are not as efficient at high dosage in saturating the body tissues as is an oil solution. The solubility of DDT in mineral oil may become a limiting factor at high levels of resistance.

In the present investigation it was impossible to kill 100 percent of the resistant strain of flies by increasing the concentration of DDT in the acetone drop applied. As a consequence the real resistance level is not known. For this reason the resistance level of the crosses to the susceptible strain appear to be much nearer that of the resistant strain than expected. This is probably a fault of the method and the true relation is not known.

In future investigations it is suggested that both speed of kill and rate of kill be recorded. If topical application is used, mineral oil or some better non-volatile liquid should be used as the solvent instead of acetone to give a truer picture of the resistance level. In addition, as pointed out by Lindquist _et al._ (1945), a lower temperature during treatment and subsequent holding would increase the mortality of treated flies. Since less concentrated solutions of DDT would be needed the difficulties encountered would be less. Temperature effects on resistance may be an important point to study as is apparent from the work reported by Johnston _et al._ (1954). In that case the method chosen should be applicable at any temperature.

SUMMARY AND CONCLUSIONS

1. Techniques for rearing single pairs of house flies were adapted from the usual rearing methods for mass cultures.

2. A strain of white-eyed flies was isolated and analyzed for method of inheritance. White eyes (w) behaved as a monofactorial character with no linkage to sex and was recessive to wild type in expression. No abnormalities in the method of inheritance in the house fly were noted during the analysis of this gene.

3. The methods used to insure proper crosses without unexpected genetical contamination were verified in the work with the white-eyed strain.

4. The manifestation of resistance to the action of DDT was shown to be inherited from both parents. There appeared to be no tendency toward sex-linkage of any major factors for resistance. The F_1 generation of reciprocal crosses was somewhere intermediate in resistance between the two parental strains. There was no significant difference in the DDT-resistance level of the F_1 generation in reciprocal crosses. No decline in resistance beyond that shown in the F_1 generation was noted in the F_2 generation. When backcrossed to the susceptible strain the resistance was lowered whether a susceptible male or female was used as a parent.

5. The Clemson strain of resistant flies proved to inherit resistance in a different manner from at least one other reported case. Since the resistance did not completely disappear in the F_2 generation

when introduced through a resistant grandfather, the theory of cytoplasmic inheritance did not apply to this strain. As knockdown time or time for death to occur were not used in this investigation, genes controlling knockdown were not evaluated. This Clemson strain did appear similar to others reported in which no clear cut evidence of simple Mendelian inheritance was found. It is indicated that resistance is inherited through the chromosomes in a manner yet to be demonstrated. The data on the Clemson strain pointed to some form of quantitative inheritance with no estimate of the number of factors involved.

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APPENDIX

Table 6. The phenotype of progeny in each generation from (white-eyed male X wild-type female).

Laboratory number	White-eyed Male	White-eyed Female	Wild-type Male	Wild-type Female
	F_1 generation = all wild-type			
	F_2 generation			
31	1	2	17	23
32	9	5	16	20
33	10	8	30	27
34	3	4	25	13
35	1	1	10	9
36	9	4	20	20
40	9	8	30	45
41	7	5	16	26
42	10	11	19	22
43	10	10	26	24
44	0	2	9	4
45	4	12	36	31
36B[a]	53	41	154	171
47[a]	75	54	203	152
Total	201	167	611	587
	Backcross to white-eyed female			
48	35	32	45	34
49	13	10	27	28
56	4	3	7	10
17[a]	147	135	162	139
Total	199	180	241	211
	Backcross to white-eyed male			
50	0	0	0	0
19[a]	157	159	148	140
Total	157	159	148	140

[a]Ten males and ten females were used as parents. All other results are from single-pair matings.

Table 7. The phenotype of progeny in each generation from (wild-type male X white-eyed female).

Laboratory number	White-eyed Male	White-eyed Female	Wild-type Male	Wild-type Female
		F_1 generation = all wild-type		
		F_2 generation		
28	7	16	47	57
29	11	21	46	48
30	1	4	9	4
37	12	15	68	48
38	11	14	45	32
39	0	0	0	0
36A[a]	72	86	290	252
46[a]	54	42	129	135
Total	168	198	634	576
		Backcross to white-eyed female		
54	8	13	4	7
55	2	7	3	2
20[a]	45	38	51	32
Total	55	58	58	41
		Backcross to white-eyed male		
51	34	8	15	16
52	3	1	2	0
53	0	0	0	0
57	17	17	21	8
21[a]	83	87	77	69
Total	137	113	115	93

[a]Ten males and ten females were used as parents. All other results are from single-pair matings.